Rec'd PCT/PTO 03 JAN 2005
PCT/EP 03/07062

12.08.2003

Europäisches Patentamt | European Patent Office | Office européen des brevets

REC'D 04 SEP 2003
WIPO PCT

Bescheinigung | Certificate | Attestation

Die angehefteten Unterlagen stimmen mit der ursprünglich eingereichten Fassung der auf dem nächsten Blatt bezeichneten europäischen Patentanmeldung überein.

The attached documents are exact copies of the European patent application described on the following page, as originally filed.

Les documents fixés à cette attestation sont conformes à la version initialement déposée de la demande de brevet européen spécifiée à la page suivante.

Patentanmeldung Nr. | Patent application No. | Demande de brevet n°

02014728.6

CERTIFIED COPY OF PRIORITY DOCUMENT

PRIORITY DOCUMENT
SUBMITTED OR TRANSMITTED IN COMPLIANCE WITH RULE 17.1(a) OR (b)

Der Präsident des Europäischen Patentamts;
Im Auftrag

For the President of the European Patent Office

Le Président de l'Office européen des brevets
p.o.

R C van Dijk

BEST AVAILABLE COPY

Europäisches Patentamt | European Patent Office | Office européen des brevets

Anmeldung Nr:
Application no.: 02014728.6
Demande no:

Anmeldetag:
Date of filing: 03.07.02
Date de dépôt:

Anmelder/Applicant(s)/Demandeur(s):

Humboldt-Universität zu Berlin
Universitätsklinikum Charite;
Akademische Verwaltung - Forschung,
Schumannstrasse 20/21
10117 Berlin
ALLEMAGNE

Bezeichnung der Erfindung/Title of the invention/Titre de l'invention:
(Falls die Bezeichnung der Erfindung nicht angegeben ist, siehe Beschreibung.
If no title is shown please refer to the description.
Si aucun titre n'est indiqué se referer à la description.)

Compound for the treatment of herpesviridae infected individuals

In Anspruch genommene Prioriät(en) / Priority(ies) claimed /Priorité(s) revendiquée(s)
Staat/Tag/Aktenzeichen/State/Date/File no./Pays/Date/Numéro de dépôt:

Internationale Patentklassifikation/International Patent Classification/
Classification internationale des brevets:

A61K38/00

Am Anmeldetag benannte Vertragstaaten/Contracting states designated at date of filing/Etats contractants désignées lors du dépôt:

AT BE BG CH CY CZ DE DK EE ES FI FR GB GR IE IT LI LU MC NL PT SE SK TR

Humboldt-Universität zu Berlin
FB11473

Compound for the treatment of herpesviridae infected individuals

Background of the invention

Herpesviridae is the name of a family of enveloped, double-strained DNA viruses with relatively large genomes. They replicate in the nucleus of a wide range of invertebrate hosts, including eight varieties isolated in humans, several each in horses, cattle, mice, pigs, chickens, turtles, lizards, fish and even in some invertebrates such as oysters. Human herpesviridae infections are endemic and sexual contact is a significant method of transmission for several including both herpes simplex virus 1 and 2(HSV-1, HSV-2, HHV1 and HHV2), also human cytomegalovirus (HCMV, HHV5) and likely Karposi's sarcoma herpesvirus (HHV-8). Four biological properties characterize members of the herpesviridae familiy:

Herpesviruses express a large number of enzymes involved in metabolism of nucleic acid (*e.g.* thymidinkinase), DNA synthesis (*e.g.* DNA helicase/primase) and processing of proteins (*e.g.* proteinkinase). The synthesis of viral genomes and the assembly of capsids occurs in the nucleus. Productive viral infection is accompanied by inevitable cell destruction. Herpesviruses are able to establish and maintain a latent state in their host and reactivate following cellular stress. Latency involves stable maintenance of the viral genome in the nucleus in the absence of any viral proteins (HSV1, HSV2, HCMV) or with limited expression of a small set of viral genes (VZV). In case of EBV latency the target cells become immortalized and transformed by expression of latency associated proteins.

The herpesviridae are divided into three sub-families (1) alpha-herpes-virinae, which includes herpes simplex virus 1(HHV1), herpes simplex virus 2(HHV2) and, varicella zoster virus (HHV3). (2) beta-herpes-virinae, which includes the human cytomegalovirus (HCMV, HHV5) and the human herpesviruses 6 and 7 (HHV6 and HHV7). (3) gamma-herpes-virinae which includes the Epstein-Barr virus (HHV4) and the Karposi's sarcoma herpesvirus (HHV8).

Herpes simplex virus 1 is responsible for facial, labial and ocular lesion. Herpes simplex virus 2 mainly for genital lesions. Varicella zoster virus is responsible for chickenpox shingles and zooster. The human cytomegalovirus (HCMV) can cause a wide varity of different diseases as

outlined in detail further below. Epstein-Barr virus is responsible for infectious mononucleosis, lympholiferative disease as well as a cofactor in human cancers (lymphomas, carcinomas). HHV8 is a cofactor in Karposi's sarcoma development which was extremely rare until the advent of AIDS.

Disease states in clinical features associated with human cytomegalovirus infection

In general, HCMV is a herpesvirus with low pathogenicity. The outcome of HCMV infection is frequently determined by the status incompetency of host immunity and, in the case of intra uterine fetal HCMV infection, the developmental stage at the time of infection. Congenital HCMV infection occurs in approximately 1% to 2% of infants *in utero* and another 6% to 60% of individuals become infected perinatally or postnatally during the first 6 months of life as the result of birth canal or breast milk transmission. The clinical features of symptomatic prenatal infections include hepatosplenomegaly, microencephaly, central nervous system disease, HCMV pneumonia, mental retardation and other symptoms.

Perinatal HCMV transmission often results from infectious uterine cervix, birth canal, milk and colostrum and other maternal reservoirs. Substantial proportions of infants (8% to 60%) become infected during the first months of life. On the other hand, perinatal infection can be symptomatic or even end in death if HCMV is transmitted to the immature *neonate*. Infections may result in symptomatic presentations such as pneumonitis, neuromuscular disability, bronchopulmonary dysplasia, and delay in speech.

HCMV infection in immuno-competent individuals is usually asymptomatic, or at the most produces a self-limited mononucleosis-like syndrome. With distinctions from Epstein-Barr virus (EBV) induced mononucleosis, HCMV mononucleosis is serologically identified to be heterophile-negative and occurs in an older age group; it is uncommon in children. The outcome of HCMV in immuno-competent patients is heavily dependent on HCMV specific protective immunities, both at the humoral and cellular levels. In congenital infection, pre-existing maternal immunity may prevent severe HCMV-induced disease.

The same is observed in organ transplant patients where the pre-existing immunity to HCMV limits diseases and syndromes associated with HCMV infection. In natural HCMV infections, immuno-competent humans respond to virus encoded envelop proteins such as capside proteins,

tegument proteins and non-structural proteins. Among them, only antibodies against viral envelope glycoproteins have functional neutralization activity.

A number of structural and non-structural HCMV proteins, particularly the major tegument protein pp65 and the immediate early protein 1 (p72), have been demonstrated to provoke helper T (T_h) and cytotoxic/suppressor T cell (T_c) responses. It has been shown that MHC class I-restricted cytotoxic T-lymphocytes displaying CD8 play an important role in host defense to HCMV infection. Cytotoxic T_c-cells recognizing the major IE proteins of HCMV are important for recovery from acute HCMV infection and for preventing reactivation of latent virus (Borysiwicz L. K. et al. (1988) Eur. J. Immunol. 18:269-275 and Eng-Shang Huang et al., The pathogenicity of human cytomegalo virus: an overview). Also, Lindsay et al. demonstrated MHC class II (DR)-restricted cytotoxicity against HCMV in the T-lymphocyte subset with the CD4 marker (helper/inducer) cells. Therefore, T_h- and T_c-lymphocytes are involved in MHC-cytotoxicity.

HCMV mononucleosis-like disease include malaise, headache, myalgia, protracted fever, abnormalities in liver functions, hepatosplenomegaly, and are typical lymphocytoses. In severe cases HCMV infections lead to the development of interstitial pneumonitis, subclinical myocarditis, pericarditis, acute and chronic encephalitis, aseptic meningitis, thrombocytopenic purpura, hemolytic anemia, gastroenteritis (colitis), hepatitis, retinitis and epidermolysis occur.

Furthermore, HCMV has the ability to infect different blood cell types mainly monocytes/macrophages. Infection of these cells usually results in persistent infections and altered expression of genes encoding cytokines and chemokines resulting in transient immuno-suppression

Other clinical manifestations of HCMV include gastrointestinal disease which is the most prominent manifestation of HCMV infection in a population of heart and heart-lung patients with an incidence of 9.9%, occurring most frequently in HCMV sero-negative recipients of organs of HCMV-sero-positive donors. Clinical manifestations include gastritis, duodenitis, esophagitis, pyloric perforation, colonic hemorrhage and more.

Clinical manifestations in immuno-compromised individuals

Populations at greatest risk of HCMV infection and HCMV-induced diseases are those undergoing organ transplantation and those with malignancies receiving immuno-suppressing chemotherapy and particularly patients with AIDS. Additionally, HCMV has been shown to cause severe complications in patients with septic disease. In immuno-compromised hosts most severe and

profound syndromes are observed when infected with HCMV, both as primary or recurring infections. Similarly, mortality and morbidity are also increased with HCMV infections in patients of this group. The most common sides of pathological involvement include adrenals (75%), lung (78%), gastrointestinal tract (30%), CNS (20%) and oculus (10%).

The severity, frequency and clinical manifestation of HCMV infections in transplant patients, cancer patients and other immuno-suppressed groups are quite variable. In most of the cases, mononucleosis fever is the common syndrome observed. After mononucleosis syndrome, pneumonia is the most frequent manifestation of HCMV infection in immuno-suppressed patients. It is more prevalent and severe in bone marrow transplant patients, with mortality rates close to 40%. Chorioretinitis is the manifestation most frequently described in association with HCMV in patients with AIDS.

Because of the ubiquitous and mysterious nature of HCMV, most of the medical problems associated with HCMV have not been adequately studied. We know that severe syndromes can result from either primary, recurring infection or superinfection with another virus strain in immuno-compromised individuals or in developing fetuses. One major public health concern is that HCMV exists commonly in human semen (and in sperm) and the cervix. It can, therefore, infect fetuses, interfere with embryonic development, and cause developmental abnormalities. Induction of latency and subsequent reactivation of HCMV is comparable to that of other oncogenic herpesviruses. One may say that HCMV infection observed today in organ transplant recipients and immuno-compromised patients are much like the visible portions of icebergs.

Infections of particular importance in transplant recipients

The most important pathogen affecting transplant recipients is HCMV, which causes both direct effects such as asymptomatic viral shedding, acute viral syndromes which are flue-like or mononucleosis-like illnesses (fever and myalgia), leucopenia, pneumonitis, infection of native tissues (retina), gastrointestinal tract (pancreas) and many more as well as indirect effects such as acute or chronic allograft rejection, immuno-suppression and more. As herpesvirus, HCMV has two properties that determine its role in transplantation: latency and cell association. Once infected (the laboratory marker of infection is sero-positivity), the patient harbors the virus for life. Activation from latency in both the recipient and the donor organ/blood is induced by many of the factors present in transplant recipients: therapy with anti-lymphocyte antibodies in cytotoxic drugs, allogeneic reactions, a systemic infection and inflammation. Thus, systemic inflammation

accompanied by the release of tumor necrosis factor and other inflammatory cytokines stimulates a variety of intracellular messengers (*e.g.* the nuclear transcription factor NF-κB), which may initiate reactivation of HCMV from latency and resulting viral replication. Replication of HCMV is highly cell-associated, with the key host defense being MHC-linked, virus specific cytotoxic T lymphocytes. Different forms of immuno-suppression used in organ transplantation affect different aspects of viral infection; anti-lymphocyte antibodies and cytotoxic drugs enhance viral activation from latency, whereas cyclosporen, tacolimus, and corticosteroids promote the persistence and spread of virus by direct effects on viral replication and by suppressing the host's antiviral immune responses. The myriad indirect effects of HCMV in transplant recipients are explained by the following observations: the virus replicates in a wide variety of cell types including epithelial cells, endothelial cells, hepatocytes, lymphocytes especially mononuclear cells and a variety of parenchymal cells. HCMV activates cellular DNA, mRNA and protein synthesis, resulting in the production of Fc receptors, intercellular adhesion molecules (vasular-cell adhesion molecules and intercellular adhesion molecules), cellular oncogenes (*myc* and *fos*), a cell-surface glycoprotein homologous II MHC class I antigens, and a variety of pro-inflammatory cytokines. These cytokines enhance the display of endothelial cell MHC class II antigens in the allograft. In addition, HCMV blocks the processing and display of HCMV specific early antigens, protecting HCMV-infected cells from cytotoxic cellular immune response. Moreover, HCMV induces production of different cytokines and chemokines causing modulation of the immune system. As a result of HCMV-mediated immune deficits, the patient is rendered more susceptible to opportunistic infections. The prevention of HCMV infection is of great importance. Although there is no consensus about the regimen of prophylaxis against HCMV three points are worth emphasizing: Firstly, the intensity of prophylaxis must be proportional to the intensity of immuno-suppression and to the risk of viral reactivation. Secondly, prophylaxis must be prevent or limitate reactivation of the virus and block HCMV replication at immediate early stage of replication to avoid pathogenic and immune modulatory effects caused by immediate early and early gene products of the virus. Thirdly, to prevent relapse after premature prophylaxis, effective anti-viral prophylaxis with negative surveillance studies must be maintained for at least three months (The New England Journal of Medicine, J. A. Fishman et al., volume 338, No. 24 pp. 1741-1751).

Chemotherapy of HCMV infection/disease knows three admitted medicaments: Ganciclovir, Foscarnet and Cidofovir. It is the main aim of therapy to hinder the establishment of HCMV disease or to reduce the severity of an HCMV infection.

Ganciclovir (GCV): Ganciclovir is a deoxyguanosine-analogue which is phosphorylated by UL97 a phosphor transferase of the virus. It is thus activated. Cellular kinases phosphorylate GCV monophosphate to di- and tri-phosphate which may serve as a competitive inhibitor of the HCMV polymerase during DNA replication, thus leads to the abortion of the DNA chain elongation. Ganciclovir is introduced either intravenously or orally.

Foscarnet (Foscavir, FCV): Foscarnet is a pyrophosphate analogue which inhibits the HCMV-polymerase by blocking the pyrophosphate binding site and thereby inhibiting the breakdown of deoxynucleosidetriphosphate to deoxynucleosidemonophosphate and pyrophosphate. The inhibition is reversible and non-competitive. In contrast to GCV, Foscarnet must not be activated and it is not incorporated into the viral DNA chain.

Cidofovir (CDV): Cidofovir is a non-cyclic nucleotide phosphonate. Cellular enzymes phosphorylate this substance to create di-phosphorylate derivative which acts as an inhibitor of the HCMV DNA-polymerase. Cidofovir-phosphonate is also incorporated into the DNA chain. This leads to termination in DNA replication.

Transplant recipients presently receive primarily GCV,whereas Foscarnet is rarely used due to its toxicity (*e.g.* nephrotoxin). Cidofovir and Foscarnet are being used for HCMV retinitis in AIDS patients. In particular for those patients which have developed a GCV resistance. Apart from the anti herpetics mentioned above, anti-HCMV-hyperimmunoglobulins and highly dosed Acyclovir is being administered to patients for prophylactic treatment. Such prophylactic treatment is being discussed controversially. In a meter analysis by Bass et al. (Bass, EB, Powe. NR, Godman, SN, Graziano, SL, Griffith, RI, Kickler, TS, Wingard, JR (1993) Efficacy of immune globuline in preventing complications of bone marrow transplantation: A meta-analysis. Bone Marrow Transplant 12:273-282) it became apparent that HCMV reactivation and infection in bone marrow recipients cannot be stopped by application of hyperimmunoglobulines.

One can conclude that blocking the viral DNA replication and late gene expression with agents such as Ganciclovir and Foscarnet would leave the infected cell relatively immune to killing by both humoral and cell-mediated factor mechanisms. In fact, virus infected cells under therapy are relatively resistant to immune attacks. The natural killer cell response against HCMV-target cells may be inhibited. Since infected "blocked" cells would not undergo the virally induced lysis associated with a productive HCMV infection, they could persist, relatively unchallenged by the immune response, during periods of antiviral treatment. HCMV infected cells treated with GCV,

FCV or CDV are able to express immediate early and early proteins known to be involved in viral pathogenesis, viral-induced immune modulation as well as immune escape of the virus-infected cell. To prevent these effects it would desirable to have therapeutics which are able to block viral replication at immediate early stage to inhibit synthesis of immediate early and early genes involved in the processes mentioned above.

In light of the state of the art, there is therefore a need for a medicament for the treatment of primarily human patients who are latently infected with HCMV and those receiving organs/cells from latently infected donors and under risk to develop severe HCMV associated disease. Ideally, this medicament would act on the latent HCMV infected cell, to prevent reactivation of the virus from latency and expression of the immediate early genes. Furthermore, there is a need for a medicament able to block HCMV replication at an immediate early (IE) or early stage of replication to avoid pathogenic, immune modulatory and immune escape effects of the virus. . To date, there is no medicament for clinical administration able to block IE and early gene expression of the virus which occurs before initiation of viral DNA replication which is inhibited by GCV, FCV or CDV. Additionally, medicaments such as Ganciclovir, Foscarnet or Cidoforvir are known to be strong cytotoxins and thus have side effects in said patients. A very recent problem is the occurrence of drug resistant HCMV strains. Resistance of HCMV to antiviral agents is a well-documented complication of long-term anti-viral therapy. This problem has been observed mostly in patients with AIDS and HCMV retinitis, in whom drug resistant HCMV infections have been associated with clinical progression and therapeutic failure. GCV resistant HCMV strains are now also observed in long-term treated transplant recipients. (Erice A. 1999. Resistance of human cytomegalovirus to antiviral drugs. Clin. Microbiol. Rev. 12:286)

The present invention provides for medicaments for the treatment of HCMV infected patients acting primarily on infected cells thereby accomplishing the maintenance of the virus in its latent state, and thus inhibiting its reactivation following, *e.g.* systemic inflammation, stress and application of cAMP-elevating drugs. Additionally, the medicament should block viral replication in permissive cells like fibroblasts, type II cells, endothelial cells, hepatocytes, smooth muscle cells to prevent pathogenic and immune modulatory effects of the virus occurring independently from viral DNA replication and production of infectious virus.

Detailed description of the invention

Monocytes (CD14+) and its undifferentiated monocyte/granulocyte progenitor cells (CD34+, CD33+) in the bone marrow have been identified as at least one main site of HCMV latency in

human (Taylor-Wiedeman et al., 1991, Mendelson et al. 1996, Kondo et al. 1996, Sindre et al., 1996 Hahn et al., 1998, Söderberg et al., 1997a). As the half-life of monocytes in the peripheral blood is restricted to 1 to 2 days, and first HCMV antigen positive monocytes are detectable not earlier than 5 to 10 days after maximum levels of plasma TNFα supports that latency occurs in progenitor cell and not in differentiated monocytes (Fietze et al., 1994, Döcke et al., 1994, Prösch et al., 1998).

Clinical studies indicated a strong correlation between TNFα plasma levels and the incidence of HCMV (re)activation (Fietze et al., 1994, Döcke et al., 1994, Mutimer et al., 1997, Kutza et al., 1998, Asadullah et al., 1999). The proposed role of TNFα for HCMV reactivation in undifferentiated monocyte/granulocyte progenitor cells was supported by the observation that TNFα can induce HCMV IE1 transcription in *in vitro* latently infected human monocyte/granulocyte progenitor cells (Hahn et al., 1998). Similarly, Hummel and co-workers (Koffron et al., 1999, Hummel et al., 2001) could induce IE1 transcription in lung cells of mice latently infected with MCMV (mouse cytomegalovirus) by application of TNFα in the absence of immuno-suppression (in mice the lung represents one organ of MCMV latency). Söderberg-Naucler and her co-workers (1997b and 1998) proposed that interferon gamma and TNFα are involved in reactivation of latent virus from monocytic cells by allogenic stimulation, however, they could not demonstrate a direct effect of TNFα on virus replication.

TNFα (via NF-κB) activation was shown to stimulate the HCMV IE1/2 enhancer/promoter in undifferentiated HL-60 cells serving as a model for monocyte/granulocyte progenitor cells in a concentration-dependent manner (Stein et al., 1993, Fietze et al., 1994). The effect was specific as it could be abrogated completely by pre-incubation of TNFα with a monoclonal antibody recognising TNFα. Using the same experimental conditions we could now show that proteasome inhibitors reduce TNFα stimulation of the IE1/2 enhancer/promoter in concentrations not cytotoxic for HL-60 cells. Moreover, proteasome inhibitors are able to reduce the basal, NF-κB-independent activity of the IE1/2 enhancer/promoter at concentrations not toxic for HL-60 cells.

More importantly, proteasome inhibitors significantly reduce HCMV replication in permissively infected embryonal lung fibroblasts representing one of the target cells of HCMV *in vivo*. Inhibition of virus replication is associated with significant decrease in IE1 and IE2 and early protein expression. Synthesis of viral late proteins, representing viral structure proteins are also significantly reduced.

The invention relates to the use of a substance or composition comprising one or more proteasome inhibitors for the manufacture of a medicament for the treatment of an individual infected with a virus selected from the group comprising varicella zoster virus, human cytomegalovirus, HHV 6 and 7, Epstein-Barr virus and HHV8.

The inventors have found out that by applying proteasome inhibitors to a progenitor-like cell line, HL-60 (ATCC No. CCL 240), it is possible to overcome TNFα-dependent stimulation of the IE1/2 enhancer/promoter. Under the same experimental conditions proteasome inhibitor 1 (PS-1) and PS-2 were tested for its influence on TNFα stimulation of the IE1/2 enhancer/promoter of HCMV AD169 and found to reduce TNFα stimulation in a concentration-dependent manner (see *e.g.* Fig. 2.).

The formulation of therapeutic compositions and their subsequent administration is believed to be within the skill in the art. In general, for therapeutics, a patient suspected of needing such therapy is given a compound and/or inhibitor in accordance with the invention, commonly in a pharmaceutically acceptable carrier, in amounts and for periods which will vary depending upon the nature of the particular disease, its severity and the patient's overall condition. The pharmaceutical compositions may be administered in a number of ways depending upon whether local or systemic treatment is desired and upon the area to be treated. Administration may be topical (including ophthalmic, vaginal, rectal, intranasal, transdermal), oral or parenteral. Parenteral administration includes intravenous drip or infusion, subcutaneous, intraperitoneal or intramuscular injection, pulmonary administration, *e.g.*, by inhalation or insufflation, or intrathecal or intraventricular administration. Formulations for topical administration may include transdermal patches, ointments, lotions, creams, gels, drops, suppositories, sprays, liquids and powders. Conventional pharmaceutical carriers, aqueous, powder or oily bases, thickeners and the like may be necessary or desirable. Coated condoms, gloves and like may also be useful. Compositions for oral administration include powders or granules, suspensions or solutions in water or nonaqueous media, capsules, sachets or tablets. Thickeners, flavouring agents, diluents, emulsifiers, dispersing aids or binders may be desirable. Compositions for parenteral, intrathecal or intraventricular administration may include sterile aqueous solutions which may also contain buffers, diluents and other suitable additives.

In addition to such pharmaceutical carriers, cationic liquids may be included in the formulation to facilitate uptake is LIPOFECTIN™ (BRL, Bethesda MD). Dosing is dependent on severity and

responsiveness of the condition to be treated, with course of treatment lasting from several days to several months or until a cure is effected or a diminution of disease state is achieved. Optimal dosing schedules can be calculated from measurements of drug accumulation in the body.

Persons of ordinary skill can easily determine optimum dosages, dosing methodologies and repetition rates. Optimum dosages may vary depending on the relative potency of individual compounds and/or inhibitors, and can generally be calculated based on IC_{50}'s or EC_{50}'s or viral infectivity levels in *in vitro* and *in vivo* animal studies. For example, given the molecular weight of a compound (derived from oligonucleotide sequence and/or chemical structure) and an effective dose such as an IC_{50}, for example (derived experimentally), a dose in mg/kg is routinely calculated.

While the substance according to the invention may be used to produce medicaments for the treatment of animals, *i.e.* mammals other than humans, humans are preferred.

Of the individuals infected with a herpesviridae virus the treatment of individuals infected with Human cytomegalovirus is preferred.

As outlined in great detail above populations at greatest risk of HCMV infection and HCMV-induced diseases are those undergoing organ transplantation, a septic disease and those with malignancies receiving immuno-suppressing chemotherapy and particularly patients with AIDS. In immuno-compromised hosts most severe and profound syndromes are observed when infected with HCMV, both as primary or recurring infections. Similarly, mortality and morbidity are also increased with HCMV infections in patients of this group. Accordingly, it in a preferred embodiment of the invention the use of the substance according to the invention is a use wherein the individuals to be treated have undergone organ transplantation are receiving immuno-suppressing chemotherapy, are otherwise immuno-suppressed or have AIDS. This may include pathological involvement of adrenals (75%), lung (78%), gastrointestinal tract (30%), CNS (20%) and oculus (10%) and consequently the use of the substance according to the invention for the manufacture of a medicament for the treatment of a pathology based on HCMV in said organs detailed above.

Proteasome inhibitors act in various ways. It is preferred here that the proteasome inhibitor is selected from a group comprising substances which are able to block the enzymatic activity of the 26S proteasome complex and/or block enzymatic activity of the 20S proteasome core structure. MG-132 *e.g.* is a cell-permeable inhibitor of the 26S proteasome which reduces the degradation of ubiquitin-conjugated proteins in mammalian cells without affecting its ATPase or isopeptidase

activities. MG-132 interferes with NF-κB activation but activates cJun N-terminal kinase (JNK1), which initiates apoptosis. Proteasome inhibitor 1 (PS-1) is a reversible inhibitor of the chymotrypsin-like activity of the multicatalytic proteinase complex 20S proteasome and has been described to prevent activation of the transcription factor NF-κB in response to TNFα. Proteasome inhibitor 2 (PS-2) also function as an inhibitor of the 20 S proteasome and NF-κB activation by blocking the decay of IκBα and IκBβ proteins. Moreover, PS-2 does not inhibit peptidyl-glutamyl-peptide hydrolyzing activity of the 20S proteasome.

In a preferred embodiment, the proteasome inhibitor is selected from a group comprising:

a) naturally occurring proteasome inhibitors comprising:
peptide derivatives which have a C-terminal expoxy keton structure, β-lacton-derivatives, aclacinomycin A, lactacystin, clastolactacystein;

b) synthetic proteasome inhibitors comprising:
modified peptide aldehydes such as N-carbobenzoxy-L-leucinyl-L-leucinyl-L-leucinal (also referred to as MG132 or zLLL), or the boric acid derivative of MG232, N-carbobenzoxy-Leu-Nva-H (also referred to as MG115), N-acetyl-L-leuzinyl-L-leuzinyl-L-norleuzinal (also referred to as LLnL), N-carbobenzoxy-Ile-Glu(OBut)-Ala-Leu-H (also referred to as PSI);

c) peptides comprising:
an α, β,-epoxyketone-structure, vinyl-sulfones such as, carbobenzoxy-L-leucinyl-L-leucinyl-L-leucin-vinyl-sulfon or, 4-hydroxy-5-iodo-3-nitrophenylacetyl-L-leucinyl-L-leucinyl-L-leucin-vinyl-sulfon (NLVS);

d) Glyoxal- or boric acid residues such as: pyrazyl-CONH(CHPhe)CONH(CHisobutyl)B$(OH)_2$ and dipeptidyl-boric-acid derivatives;

e) Pinacol-esters such as: benzyloxycarbonyl(Cbz)-Leu-leuboro-Leu-pinacol-ester.

In another preferred embodiment, the proteasome inhibitor is selected from a group comprising:

a) epoxomicin ($C_{28}H_{86}N_4O_7$) and/or

b) eponemycin ($C_{20}H_{36}N_2O_5$).

In just another preferred embodiment, the proteasome inhibitor is selcted from a group comprising:

a) PS-314 as a peptidyl-boric-acid derivative which is N-pyrazinecarbonyl-L-phenylalanin-L-leuzin- boric acid ($C_{19}H_{25}BN_4O_4$);

b) PS-519 as a β-lacton- and a lactacystin-derivative which is 1R-[1S, 4R, 5S] -1-(1-Hydroxy-2methylpropyl)-4-propyl-6-oxa-2azabicyclo[3.2.0]heptane-3,7-dione ($C_{12}H_{19}NO_4$);

c) PS-273 (morpholin-CONH-(CH-naphthyl)-CONH-(CH-isobutyl)-B(OH)$_2$) and its enantiomere;

d) PS-293;

e) PS-296 (8-quinolyl-sulfonyl-CONH-(CH-napthyl)-CONH(-CH-isobutyl)-B(OH)$_2$);

f) PS-303 (NH$_2$(CH-naphthyl)-CONH-(CH-isobutyl)-B(OH)$_2$;

g) PS-321 as (morpholin-CONH-(CH-napthyl)-CONH-(CH-phenylalanin)-B(OH)$_2$);

h) PS-334 (CH$_3$-NH-(CH-naphthyl-CONH-(CH-Isobutyl)-B(OH)$_2$);

i) PS-325 (2-quinol-CONH-(CH-homo-phenylalanin)-CONH-(CH-isobutyl)- B(OH)$_2$;

j) PS-352 (phenyalanin-CH$_2$-CH$_2$-CONH-(CH-isobutyl)l-B(OH)$_2$;

k) PS-383 (pyridyl-CONH-(CH$_p$F-phenylalanin)-CONH-(CH-isobutyl)-B(OH)$_2$);

l) PS-341; and

m) PS-1 Z-Ile-Glu(O*t*Bu)-Ala-Leu-CHO;

PS-2 [Benzyloxycarbonyl)-Leu-Leu-phenylalaninal or Z-LLF-CHO or Z-Leu-Leu-Phe-CHO PS-1.

In another preferred embodiment, the proteasome inhibiors is selected from a group comprising:

a) PS-341 and

b) PS-1 Z-Ile-Glu(O*t*Bu)-Ala-Leu-CHO;
PS-2 [Benzyloxycarbonyl)-Leu-Leu-phenylalaninal or Z-LLF-CHO or Z-Leu-Leu-Phe-CHO PS-1.

c) PS-519 as a β-lacton- and a lactacystin-derivative which is 1R-[1S, 4R, 5S]-1-(1-Hydroxy-2methylpropyl)-4-propyl-6-oxa-2azabicyclo[3.2.0]heptane-3,7-dione ($C_{12}H_{19}NO_4$)

Further proteasome inhibitors according to the invention are also listed in Table 1.

The present invention also encompasses a method of treating or preventing a viral infection in a subject, the method comprising administering to the subject an amount of a compound or substance selected from the group comprising proteasome inhibitors, wherein the viral infection is an infection of varicella zoster virus, human cytomegalovirus, human herpesvirus 6 and 7, Epstein-Barr virus and Karposi's sarcoma herpesvirus.

While the inventors believe the substance according to the invention may be used to treat animals, i.e. mammals other than humans, humans are preferred.

The treatment is performed with a proteasome inhibitor selected from a group comprising:
naturally occurring proteasome inhibitors comprising:
peptide derivatives which have a C-terminal expoxy keton structure, β-lacton-derivatives, aclacinomycin A, lactacystin, clastolactacystein;

synthetic proteasome inhibitors comprising:
modified peptide aldehydes such as N-carbobenzoxy-L-leucinyl-L-leucinyl-L-leucinal (also referred to as MG132 or zLLL or Z-Leu-Leu-Nva-CHO), or the boric acid derivative of MG132, N-carbobenzoxy-Leu-Nva-H (also referred to as MG115), N-acetyl-L-leuzinyl-L-leuzinyl-L-

norvalinal (also referred to as Z-LL-Nva-CHO or Z-Leu-Leu-Nva-CHO), N-carbobenzoxy-Ile-Glu(O*t*But)-Ala-Leu-H (also referred to as PSI);

peptides comprising:
an α, β,-epoxyketone-structure, vinyl-sulfones such as, carbobenzoxy-L-leucinyl-L-leucinyl-L-leucin-vinyl-sulfon or, 4-hydroxy-5-iodo-3-nitrophenylacetyl-L-leucinyl-L-leucinyl-L-leucin-vinyl-sulfon (NLVS);
Glyoxal- or boric acid residues such as: pyrazyl-CONH(CHPhe)CONH(CHisobutyl)B$(OH)_2$ and dipeptidyl-boric-acid derivatives;

Pinacol-esters such as: benzyloxycarbonyl(Cbz)-Leu-leuboro-Leu-pinacol-ester;
epoxomicin ($C_{28}H_{86}N_4O_7$) and eponemycin ($C_{20}H_{36}N_2O_5$);
PS-314 as a peptidyl-boric-acid derivative which is N-pyrazinecarbonyl-L-phenylalanin-L-leuzin-boric acid ($C_{19}H_{25}BN_4O_4$);
PS-519 as aβ-lacton- and a lactacystin-derivative which is 1R-[1S, 4R, 5S] -1-(1-Hydroxy-2methylpropyl)-4-propyl-6-oxa-2azabicyclo[3.2.0]heptane-3,7-dione ($C_{12}H_{19}NO_4$);
PS-273 (morpholin-CONH-(CH-naphthyl)-CONH-(CH-isobutyl)-B$(OH)_2$) and its enantiomere PS-293;
PS-296 (8-quinolyl-sulfonyl-CONH-(CH-napthyl)-CONH(-CH-isobutyl)-B$(OH)_2$);
PS-303 (NH_2(CH-naphthyl)-CONH-(CH-isobutyl)-B$(OH)_2$;
PS-321 as (morpholin-CONH-(CH-napthyl)-CONH-(CH-phenylalanin)-B$(OH)_2$);
PS-334 (CH_3-NH-(CH-naphthyl-CONH-(CH-Isobutyl)-B$(OH)_2$);
PS-325 (2-quinol-CONH-(CH-homo-phenylalanin)-CONH-(CH-isobutyl)- B$(OH)_2$;
PS-352 (phenyalanin-CH_2-CH_2-CONH-(CH-isobutyl)l-B$(OH)_2$;
PS-383 (pyridyl-CONH-(CH_pF-phenylalanin)-CONH-(CH-isobutyl)-B$(OH)_2$);
PS-341 and
PS-1 Z-Ile-Glu(O*t*Bu)-Ala-Leu-CHO.
PS-2 [Benzyloxycarbonyl)-Leu-Leu-phenylalaninal or Z-LLF-CHO or Z-Leu-Leu-Phe-CHO.

Preferred proteasome inhibitors may be selected from the group comprising: a) PS-341 and b) PS-1 Z-Ile-Glu(O*t*Bu)-Ala-Leu-CHO and c) PS-519 as a β-lacton- and a lactacystin-derivative which is 1R-[1S, 4R, 5S] -1-(1-Hydroxy-2methylpropyl)-4-propyl-6-oxa-2azabicyclo[3.2.0]heptane-3,7-dione ($C_{12}H_{19}NO_4$).

The invention further relates to a method of treating or preventing a viral infection in a subject, the method comprising administering to the subject an amount of a compound or substance selected from the group comprising proteasome inhibitors, wherein the viral infection is an infection of varicella zoster virus, cytomegalovirus, human cytomegalovirus, human herpesvirus 6 and 7, Epstein-Barr virus and Karposi's sarcoma herpesvirus. Incorporated herein are all embodiments concerning the use of a substance or composition comprising one or more proteasome inhibitors for the manufacture of a medicament as outlined in detail above.

Examples

Example 1

Influence of proteasome inhibitor MG-132 on TNFα stimulation of the HCMV IE1/2 enhancer/promoter in HL-60 cells

As a model for undifferentiated monocyte/granulocyte progenitor cells HL-60, cells (ATCC No. CCL 240) expressing high levels of CD34+ but low levels of typical differentiation antigens like CD11a-c and CD14 on their surface were used. Furthermore, HL-60 cells have retained the ability to differentiate into granulocytes and monocytes depending on the stimulus. HL-60 cells were grown in RPMI 1640 medium supplemented with 10 % fetal calf serum (both certified endotoxin-free, Biochrome, Germany) at 37°C in a 5 % humidified atmosphere. The cells were shown to be mycoplasma-free by the Mycoplasma Detection Kit (Boehringer Mannheim, Germany). The cells were grown up to $1x10^6$ cells per ml. HL-60 cells were transiently transfected with the plasmid pRR55 containing the native HCMV strain Ad169 IE1/2 enhancer/promoter region between nucleotides –671 and +52 relative to the transcription start site upstream of the chloramphenicol acetyl transferase (CAT) reporter gene (Fickenscher et al., 1989). Plasmid DNA was prepared using the endofree Maxi Kit (QUIAGEN, Germany or Machery & Nagel, Germany) and stored in aliquots at –20°C. Transient transfection was performed using the DEAE transfection protocol (Stein et al., 1993). Per transfection reaction $5x10^5$ cells were washed once with FCS-free medium and then resuspendend in 250 µl transfection buffer containing 1xTBS (24 mM Tris-HCl, pH 7.4, 137 mM NaCl, 5 mM KCl, 0.7 mM $CaCl_2$, 0.5 mM $MgCl_2$, 0.6 mM Na_2HPO_4), 2.5 µg plasmid DNA (1µg/µl) and 5 mg/ml DEAE dextran (Pharmacia-Amersham, Germany). To avoid differences in transfection efficiency, cells for parallel probes were transfected in one reaction and splitted after transfection. Transfection occurred for 1 h at 37 °C. After that the cells were washed once with FCS-free RPMI 1640 medium and resuspended in medium containing 10 % FCS at a concentration of $5x10^5$ cells/5ml. MG-132 (Calbiochemie, Germany) was dissolved in ethanol (5µg/µl) and stored in aliqotes at –20 °C. MG-132 was added into the medium at time zero after transfection and 1 h before TNFα (5 ng/ml, human recombinant TNFα; PAN, Germany, stored in aliquots containing 10µg/µl at –80°C). The cells were harvested 48 h after transfection, washed once with PBS and resuspended in 70 µl CAT buffer 1 (0.25 M Tris/HCl, pH 7.8, 0.5 mM EDTA). After 10 min of incubation on ice, cell extracts were prepared by repeated frozen/thawing of the

cells (routinely 5 times), 10 min incubation at 65 °C to inactivate isoenzymes. Cell debris was removed by centrifugation at 12 000 rpm for 10 min in an Eppendorf centrifuge. Cell extracts were measured for their protein concentrations using the method of Bradford (Bradford, 1976) and Bradford reagent (Sigma, Germany). Equal quantities of protein were used in the CAT-assay as described by Gorman et al., 1982. 20 µl lysates were incubated with [^{14}C]chloramphenicol (Hartman Analytic, Germany) and acetyl-CoA (Sigma, Germany) at 37 °C. Reaction was stopped by extraction with ethylacetat (Merck, Germany). The acetylated products were quantified after separation by thin-layer chromatography using a thin layer scintillator. MG-132 in concentration of 0.5 µg/ml abrogated the TNFα dependent stimulation of the IE1/2 enhancer/promoter nearly completely. In the presence of 0.1 µg/ml MG-132 reduced TNFα stimulation by 50 % (Fig.1). At these concentrations no cytotoxic effects on HL-60 cells were observed. MG-132 in concentrations above 0.5 µg/ml also inhibited basic, TNFα-independent activity of the IE1/2 enhancer/promoter in HL-60 cells (Fig.10).

Example 2

Effect of proteasome inhibitor PS-1 on TNFα stimulation of the HCMV IE1/2 enhancer/promoter in HL-60 cells

Under the same experimental conditions proteasome inhibitor 1 (PS-1, Calbiochemie, Germany) and PS-2 were tested for its influence on TNFα stimulation of the IE1/2 enhancer/promoter of HCMV AD169 and found to reduce TNFα stimulation in a concentration-dependent manner. The results for PS-1 are summarised in Fig. 2.

Examples 3 – 6

Effect of proteasome inhibitors MG-132 and PS-1 on HCMV replication in human embryonal lung fibroblasts (Fi 301)

Human embryonal lung fibroblasts (HELF), one of the target cells of HCMV *in vivo*, are fully permissive for HCMV replication *in vitro*. For infection experiments the laboratory adapted strain AD169 was propagated on HELFs. Virus stocks prepared from the overlay of infected cells showing 100% cytopathic effect (CPE) by ultra centrifugation were stored in liquid N_2. Confluent HELF monolayer in 50 cm^2 flasks were infected with AD169 at a multiplicity of infection (M.O.I.)

of 0.01. Adsorption of the virus was allowed for 1 hour at 37°C. After that the monolayer was overlayed with MEM containing 4.5% FCS. Medium was free of or substituted with the proteasome inhibitor at the indicated concentration. Virus cultures were cultivated for 5 days without changing the medium. On day five *p.i.* virus replication was quantified by the number of CPE visible in inverse light microscope. For quantification cells were stained with an HCMV-specific antibody recognising the IE proteins IE1 and IE2 (clone E13, Harlan Sera-Lab, Loughborough, GB, 1:5000), goat anti-mouse HRP-conjugated IgG (1:4000; Boehringer Mannheim, Indianapolis, IN, USA) as secondary antibody and the AEC staining kit (Sigma, Germany) which gives a reed nuclear staining of infected cells.

As shown in Fig. 3 MG-132, a concentration of 0.2 µg/ml, which is non-toxic for HELFs reduces the number of CPE by two orders of magnitudes. PS-1 under the same conditions dropped virus replication by 1 to 2 order of magnitudes (not shown). Inhibition of virus replication is documented by a significantly reduced number of virus-infected cells per focus (50 vs. 7 infected cells) indicating that MG132 blocks virus spread (Fig. 4 and 5)

Example 7

Proteasome inhibitor MG-132 had no effect on herpes simplex type 1 (HSV1, HHV1) and herpes simplex type 2 (HSV2, HHV2) replication in human embryonal lung fibroblasts

Confluente HELF monolayers in 24 well tissue culture plates were infected with HSV1 strain K or HSV2 at a M.O.I. of 0.001. Virus adsorption was allowed for 1 h at 37°C. After removing the viral inoculate, cells were overlayed with MEM/E, 1% Methocel and incubated for two days at 37 °C in a 5 % CO_2 atmosphere. MG-132 at the indicated concentrations was added into the medium after adsorption. CPEs were quantified by light microscopy. MG132 had no effect on HSV1 (Fig. 6) and HSV2 (not shown) replication in HELF.

Example 8

MG-132 partially inhibits IE1/2 gene expression as well as early and late gene expression in HCMV AD169-infected HELF

HELF were infected with AD169 at a M.O.I. of 1 and incubated as descibed in example 3-6. MG-132 at a concentration of 0.2 µg/ml was added after adsorption of the virus.

Cells were harvested at indicated day, washed 2 times with PBS, solubilized in 200 µl lysis buffer containing 20 mM Tris-HCL, pH 7.5, 150 mM NaCl, 1 % NP40, 0.02 % NaN_3, aprotinin (1 µg/ml), antipain (1 µg/ml), leupeptin (2 µg/ml), phenyl-methyl-sulfonyl fluoride (PMSF) (2 mM) (all inhibitors from Roche, Mannheim, Germany) and 2.5 mM EDTA, shaken for 1h at 350 rpm and centrifuged for 50 min at 18,000 g and 4°C to remove lipids and cell debris. The protein-containing supernatant was stored at -70 °C. Protein quantification was performed using Advanced Protein Assay Reagent (TEBU, Frankfurt, Germany). Per lane, 80 µg protein denatured for 5 min. at 95 °C were electrophoresed on a 7.5% sodium dodecyl sulfate (SDS)-polyacrylamid gel and transferred (Mini Tank Elektroblotter, OWLScientific, USA) to a cellulosenitrate membrane (Protran BA 85, Schleicher & Schuell, Dassel, Germany). The membrane was blocked overnight in TBST (10 mM Tris HCl, pH 7.4, 150 mM NaCl, 0.05 % Tween 20) supplemented with 3 % FCS. Membranes were incubated with either, mouse anti-HCMV IE antigen (recognising IE1 and IE2 protein), clone E13 (1:500; Harlan Sera-Lab, Loughborough, GB), mouse monoclonal anti-HCMV p68 late protein (1:5,000; Advanced Biotechnology, Columbia, Maryland, USA) or goat anti-actin antibody (1:1,000; SantaCruz Biotechnology Inc., Heidelberg, Germany). After five times washing with TBST, the blots were incubated with the second, anti-species specific antibody; goat anti-mouse HRP-conjugated IgG (1:4000; Boehringer Mannheim, Indianapolis, IN, USA) or donkey anti-goat HPR-conjugated IgG (1:4000; SantaCruz Biotechnology, Heidelberg, Germany) for 1h. The immunoreactive bands were visualized and quantified using SuperSignal substrate (Pierce, Rockford, IL, USA) and a CCD-Camera (Raytest, Germany).

Blots shown in Fig. 8 demonstrate that IE protein expression in MG-132 cells is reduced compared with the untreated virus control. Remarkably, the effect is stronger with respect to IE2 than to IE1 protein. Late protein expression (p68) is completely blocked in MG-132-treated cells until day 4 p.i. while actin levels remained unchanged.

Figures Captions

Figure 1

Figure 1 shows results from Example 1 (means ± SEM) of four independent experiments (see above). MG-123 decreased TNFα stimulation of the IE1/2 enhancer/promoter in a concentration-dependent manner between 0.5 and 0.05 μg/ml (0.81 to 0.081 μM). The IC_{50} was determined between 0.1 and 0.2 μg/ml (0.21 to 0.42 μM).

Figure 2

Concentrations of 0.5 to 0.1 μg/ml (0.81 to 0.16 μM) PS-1 decreases TNFα stimulation by about 60 %. The IC_{50} was determined between 0.2 and 0.3 μM in four independent experiments.

Figure 3

In the presence of 0.2 μg/ml (0.42 μM) MG-132 virus replication was reduced by two orders of magnitudes, addition of 0.1 μg/ml (0.21 μM) MG-132 decreased virus replication by about one order of magnitude, while 0.01 μg/ml (0.016 μM) MG-132 had no effect (See also Example 3-6).

Figure 4

MG-132 inhibits spread of the virus. In the cultures containing 0.2 μg/ml (0.42 μM) MG-123 mainly single infected cells or very small foci containing between 2 and 7 IE-positive cells were observed, indicating that virus spread in cell culture was significantly inhibited. The number of IE-positive cells per foci in cultures grown in the presence of 0.1 μg/ml (0.21 μM) MG-132 were about 50 % compared with the untreated control.

Figure 5

Representative pictures from AD169-infected cell cultures untreated (A) or MG-132 treated (B-D) at day 5 p.i. are shown. Virus infected cells are stained using the IE1/2 specific antibody clone E13.

A: untreated virus control

B: 0.2 µg/ml MG-132

C: 0.1 µg/ml MG-132

D: 0.001 µg/ml MG-132

Figure 6

Under the same experimental conditions as described in example 3-6 PS-1 inhibits HCMV AD169 replication also by 1 to 2 orders of magnitude. The ID_{50} was determined between 0.16 and 0.08 µM.

Figure 7

MG-132 at concentrations between 0.2 and 0.01 µg/ml (0.42 to 0.021 µM) had no effect on replication of HSV 1 in HELF .

Figure 8

Fig. 8 shows Western Blot analysis of cells (HELF) infected with HCMV AD169 and grown in the absence or presence of MG-132 (0.175 µg/ml). Blots shown demonstrate that (A) IE 1 and IE2 protein expression in MG-132 treated cells is reduced compared with the untreated virus control. Late protein expression (p68) is reduced and completely absent at day 3 p.i., while actin levels remained unchanged.

(M.O.I. =1) at days 1, 3, 5 and 7 *p.i.* and grown in the absence or presence of MG-132 (0.175 µg/ml) (see also Experiment 8). At days 3 and 5 medium was changed and supplemented with MG-132.

Figure 9

Figure 9 shows the chemical structure of PS-341 and PS-519

Figure 10

MG-132 at concentrations above 0.5 µg/ml inhibits basal activity of the HCMV IE1/2 enhancer/promoter in HL-60 cells.

Table 1 Proteasome inhibitors Molecule	References
Proteasome inhibitors	
Peptide Aldehydes:	Palombella et al., 1994; Grisham et al., 1999; Jobin et al., 1998a
ALLnL (N-acetyl-leucinyl-leucynil-norleucynal, MG101)	
LLM (N-acetyl-leucinyl-leucynil-methional)	
Z-LLnV (carbobenzoxyl-leucinyl-leucynil-norvalinal,MG115)	Lopes et al., 1997. J. Biol. Chem. 272:12893; Lee and Goldberg. 1996. J. Biol. Chem. 271:27280; Palombella et al., 1994. Cell 78:773; Rock et. al. 1994. Cell 78:761; Vinitski et al., 1992. Biochemistry 31:9421
Z-LLL (carbobenzoxyl-leucinyl-leucynil-leucynal, MG132)	Steinhib et al., 2001. J. Biol. Chem.276:4476; Merlin et al., 1998. J. Biol. Chem. 273:6373; Adams and Stein, 1996. Ann. Rev. Med. Chem. 31:279; Klafki et al., 1996. J. Biol. Chem. 271:28655; Lee and Goldberg, 1996; Wiertz et al., 1996. Cell 84:769; Jensen et al., 1995. Cell 83:129; read et al., 1995. Immunity 2:493; Rock et al., 1994
Lactacystine, b-lactone	Fenteany et al., 1998; Grisham et al., 1999
Boronic Acid Peptide	Grisham et al., 1999; Iqbal et al., 1995
Ubiquitin Ligase Inhibitors	Yaaron et al., 1997
PS-341	Adams, 2001
Cyclosporin A	Frantz et al., 1994; Kunz et al., 1995; Marienfield et al., 1997; McCaffrey et al. 1994; Meyer et al., 1997; Wechsler et al., 1994
FK506 (Tacrolimus)	Okamoto et al., 1994; Venkataraman et al., 1995
Deoxyspergualin	Tepper et al., 1995

EPO - Munich
33
03 Juli 2002

Humboldt-Universität zu Berlin
FB11473

Claims

1. Use of a substance or composition comprising one or more proteasome inhibitors for the manufacture of a medicament for the treatment of an individual infected with a virus selected from the group comprising varicella zoster virus, human cytomegalovirus, HHV6 and 7, Epstein-Barr virus and HHV8.

2. Use of a substance according to claim 1, wherein the individual is a human and the virus is human cytomegalovirus.

3. Use of a substance according to claims one or two, wherein the individual has undergone organ transplantation, is receiving immuno-suppressing chemotherapy, is otherwise immuno-suppressed, has a septic disease or has AIDS.

4. Use of a substance according to any of the preceeding claims, wherein the proteasome inhibitor is selected from a group comprising substances which are able to block the enzymatic activity of the 26S proteasome complex and/or block enzymatic activity of the 20S proteasome core structure.

5. Use of a substance according to any of the preceeding claims, wherein the proteasome inhibitor is selected from a group comprising:

   a) naturally occurring proteasome inhibitors comprising:
   peptide derivatives which have a C-terminal expoxy keton structure, β-lacton-derivatives, aclacinomycin A, lactacystin, clastolactacystein;

   b) synthetic proteasome inhibitors comprising:
   modified peptide aldehydes such as N-carbobenzoxy-L-leucinyl-L-leucinyl-L-leucinal (also referred to as MG132 or zLLL), or the boric acid derivative of MG232, N-carbobenzoxy-Leu-Nva-H (also referred to as MG115), N-acetyl-L-leuzinyl-L-leuzinyl-L-norleuzinal (also referred to as LLnL), N-carbobenzoxy-Ile-Glu(OBut)-Ala-Leu-H (also referred to as PSI);

   c) peptides comprising:

an α, β,-epoxyketone-structure, vinyl-sulfones such as, carbobenzoxy-L-leucinyl-L-leucinyl-L-leucin-vinyl-sulfon or, 4-hydroxy-5-iodo-3-nitrophenylacetyl-L-leucinyl-L-leucinyl-L-leucin-vinyl-sulfon (NLVS);

d) Glyoxal- or boric acid residues such as: pyrazyl-CONH(CHPhe)CONH(CHisobutyl)$B(OH)_2$ and dipeptidyl-boric-acid derivatives;

e) Pinacol-esters such as: benzyloxycarbonyl(Cbz)-Leu-leuboro-Leu-pinacol-ester.

6. Use of a substance according to claim 4 wherein the proteasome inhibitor is selected from a group comprising:

a) epoxomicin ($C_{28}H_{86}N_4O_7$) and/or

b) eponemycin ($C_{20}H_{36}N_2O_5$).

7. Use of substance according to claim 4, wherein the proteasome inhibitor is selected from a group comprising:

a) PS-314 as a peptidyl-boric-acid derivative which is N-pyrazinecarbonyl-L-phenylalanin-L-leuzin- boric acid ($C_{19}H_{25}BN_4O_4$);

b) PS-519 as a β-lacton- and a lactacystin-derivative which is 1R-[1S, 4R, 5S] -1-(1-Hydroxy-2methylpropyl)-4-propyl-6-oxa-2azabicyclo[3.2.0]heptane-3,7-dione ($C_{12}H_{19}NO_4$);

c) PS-273 (morpholin-CONH-(CH-naphthyl)-CONH-(CH-isobutyl)-$B(OH)_2$) and its enantiomere;

d) PS-293;

e) PS-296 (8-quinolyl-sulfonyl-CONH-(CH-napthyl)-CONH(-CH-isobutyl)-$B(OH)_2$);

f) PS-303 (NH_2(CH-naphthyl)-CONH-(CH-isobutyl)-B$(OH)_2$;

g) PS-321 as (morpholin-CONH-(CH-napthyl)-CONH-(CH-phenylalanin)-B$(OH)_2$);

h) PS-334 (CH_3-NH-(CH-naphthyl-CONH-(CH-Isobutyl)-B$(OH)_2$);

i) PS-325 (2-quinol-CONH-(CH-homo-phenylalanin)-CONH-(CH-isobutyl)-B$(OH)_2$;

j) PS-352 (phenyalanin-CH_2-CH_2-CONH-(CH-isobutyl)1-B$(OH)_2$;

k) PS-383 (pyridyl-CONH-(CH_pF-phenylalanin)-CONH-(CH-isobutyl)-B$(OH)_2$);

l) PS-341; and

m) PS-1 Z-Ile-Glu(O*t*Bu)-Ala-Leu-CHO;
PS-2 [Benzyloxycarbonyl)-Leu-Leu-phenylalaninal or Z-LLF-CHO or Z-Leu-Leu-Phe-CHO PS-1.

8. Use of a substance according to claim 7, wherein the substance is selected from the group comprising:

a) PS-341 and

b) PS-1 Z-Ile-Glu(O*t*Bu)-Ala-Leu-CHO;
PS-2 [Benzyloxycarbonyl)-Leu-Leu-phenylalaninal or Z-LLF-CHO or Z-Leu-Leu-Phe-CHO PS-1.

c) PS-519 as a β-lacton- and a lactacystin-derivative which is 1R-[1S, 4R, 5S]-1-(1-Hydroxy-2methylpropyl)-4-propyl-6-oxa-2azabicyclo[3.2.0]heptane-3,7-dione ($C_{12}H_{19}NO_4$)

Humboldt-Universität zu Berlin
FB11473

EPO - Munich
33
03. Juli 2002

Abstract

The present invention relates to the use of a substance or composition comprising one or more proteasome inhibitors for the manufacture of a medicament for the treatment of an individual infected with a virus selected from the group comprising varicella zoster virus, human cytomegalovirus, human herpesvirus 6 and 7 and Epstein-Barr virus and Karposi's sarcoma herpesvirus. The invention further relates to methods of treatment of individuals infected with a virus selected from the group comprising varicella zoster virus, human cytomegalovirus, human herpesvirus 6 and 7 and Epstein-Barr virus and Karposi's sarcoma herpesvirus.

Figures

Figure 1

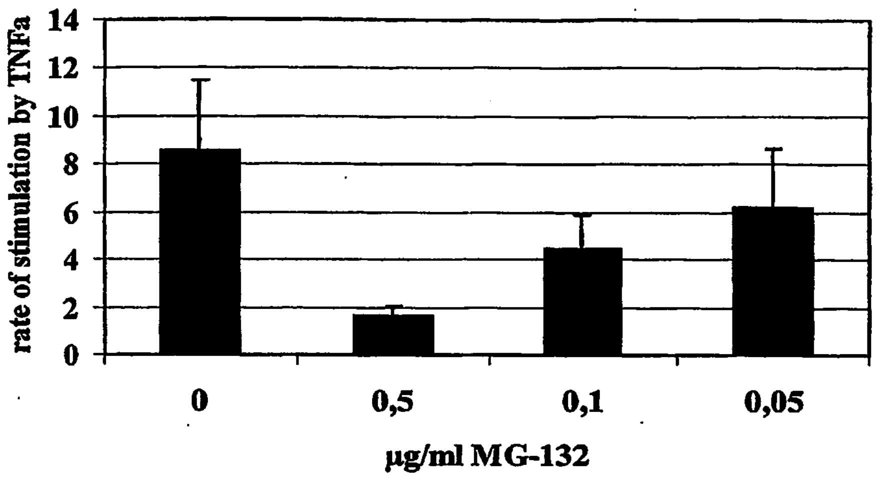


Figure 2

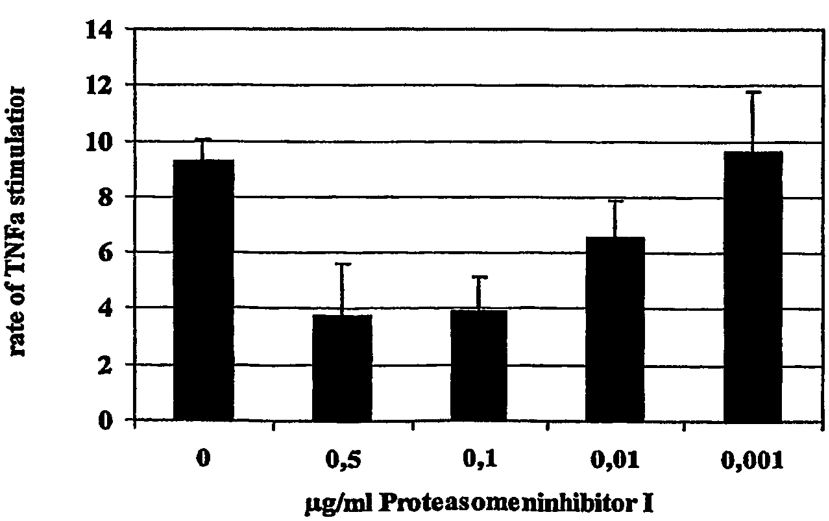


Figure 3

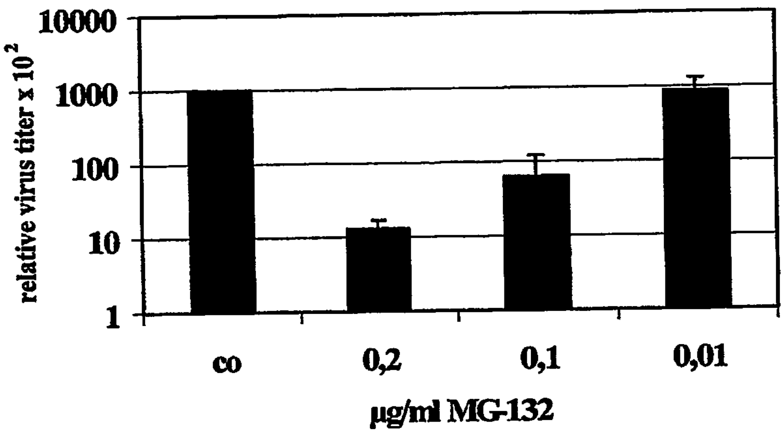


Figure 4

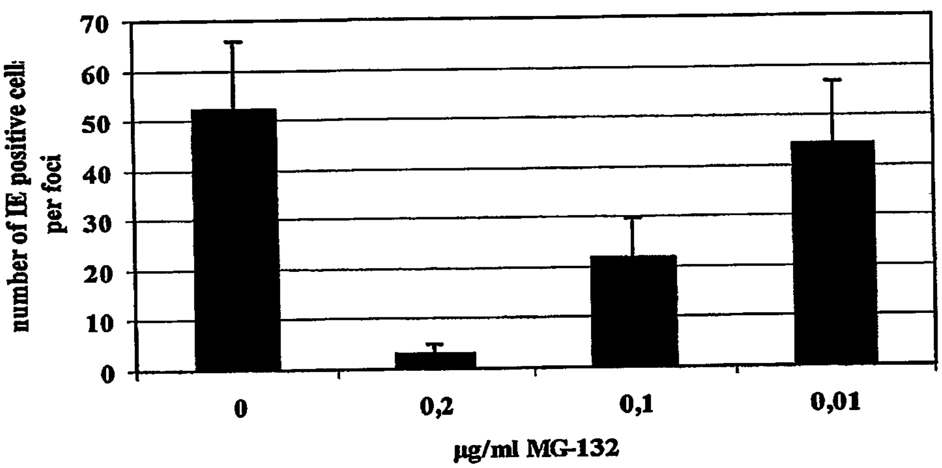
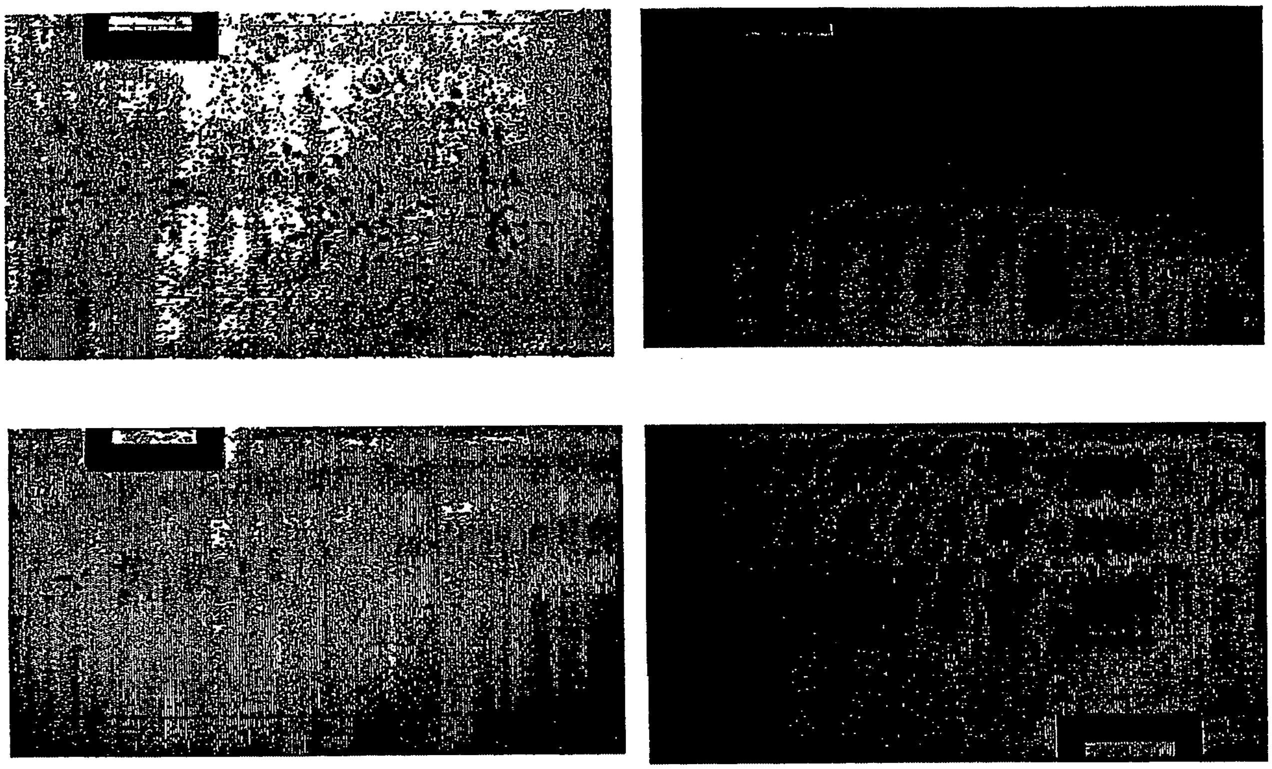


Figure 5

A B



C D

Figure 6

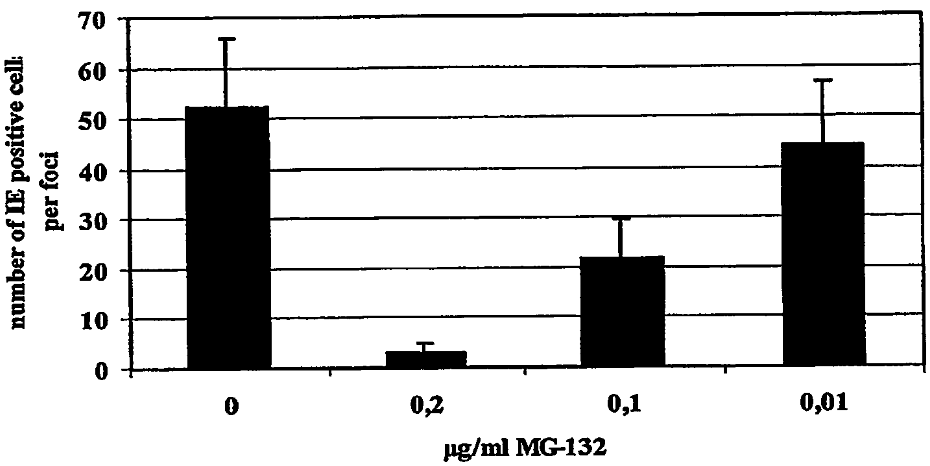


Figure 7

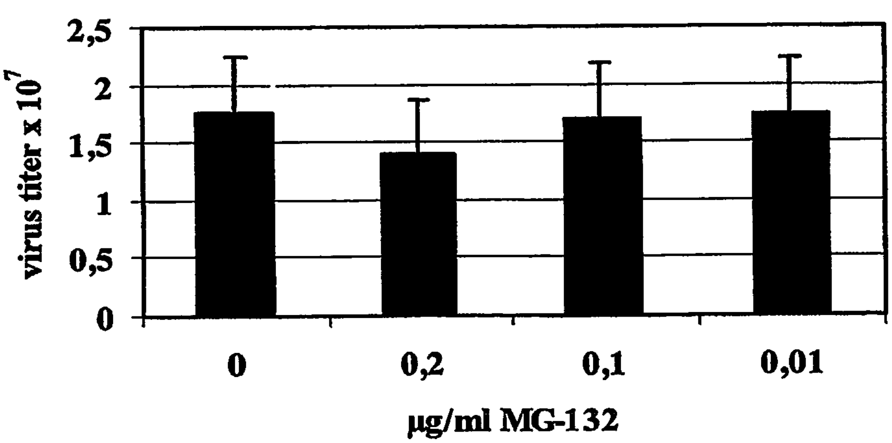


Figure 8

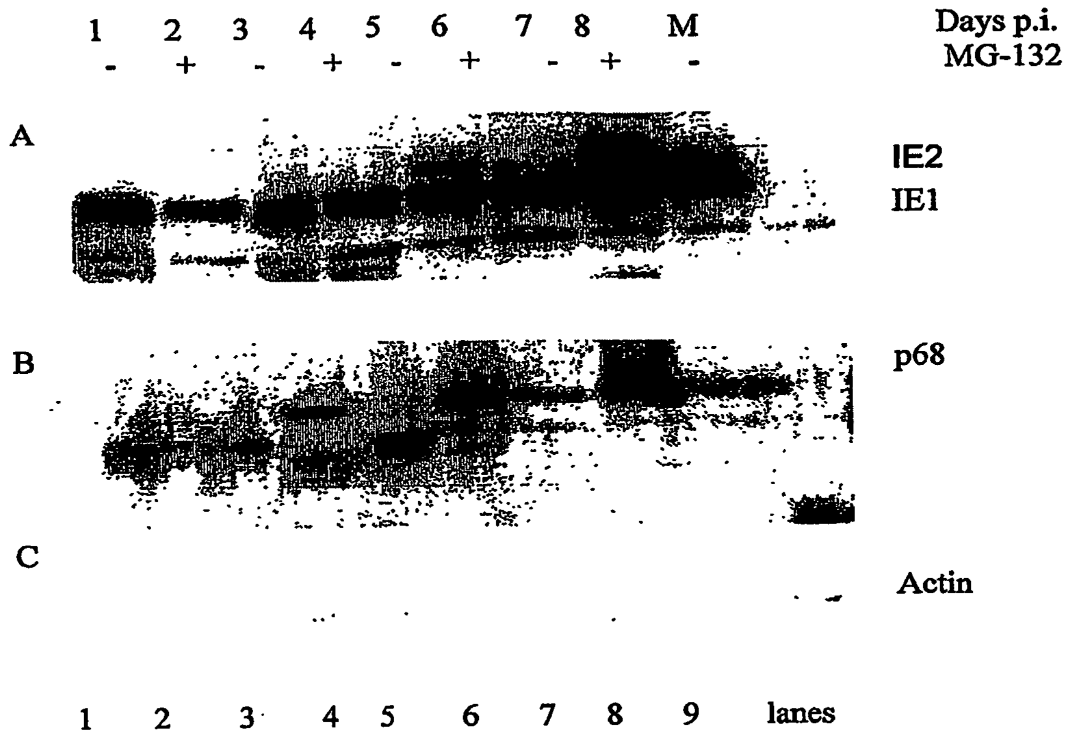
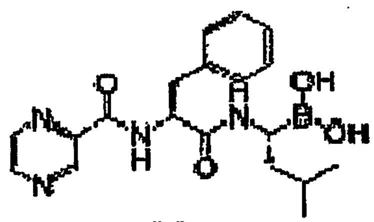
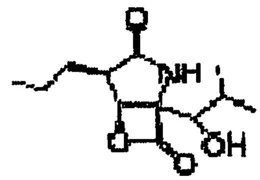

1 2 3 4 5 6 7 8 M
Days p.i.
- + - + - + - + -
MG-132
A
IE2
IE1
B
p68
C
Actin
1 2 3 4 5 6 7 8 9 lanes

Figure 9



PS-341



PS-519

Figure 10